REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested. Pursuant to 37 CFR § 1.121, attached as Appendix A is a Version With Markings to Show Changes Made.

According to recent estimates, the global demand for crop plants such as rice, wheat, and maize should increase by 40% by 2020. It is thought that classical plant breeding technology, which led to the green revolution in the late 1960s, will contribute less and less to meet this increasing demand, whereas plant genetic engineering will contribute increasingly more. An important thrust area in plant genetic engineering is the identification and use of genes implicated in asexual production of seeds, or "apomixis." Apomixis is thought to be an agronomically desirable trait that should enable seed companies and farmers to lock-in a favorable combination of genes for maximum grain yield without having to lose the gene combination in the next sexual generation. Genes for apomixis have not yet been identified. It is thought that genes that are generally important for very early embryo/seed development may be important for apomixis. A second important thrust is the production of early flowering varieties of plants such that breeding time can be reduced.

The evolution of flowering plants may have entailed a modification of primitive leaf or leaf-like structures that contained naked ovules on their surfaces, to specify floral organs that ultimately evolved to surround the ovules. This view of angiosperm evolution predicts that the genetic regulatory network that controls ovule development should be interlaced with that which triggers flowering. Ovule, as the precursor of seed, is the link to the next generation. Genetic regulatory pathways that are important for early vegetative development of the embryo inside the ovule, for late reproductive development leading to the production of ovules, and for morphogenesis of the haploid female gametophyte, are crucial areas of investigation which can lead to enhanced agricultural practices.

Several genes important for ovule development have been identified in *Arabidopsis thaliana*. *BELL1*, a so-called cadastral gene that encodes a homeodomain protein, controls the expression of the floral organ identity gene *AG* within the ovule and thereby controls morphogenesis of ovule integuments. *SUPERMAN*, another cadastral gene that restricts the spatial expression pattern of the floral organ identity gene *AP3*, is important in ovule integument development. The organ identity gene *AP2* is also known to control

ovule morphogenesis. By contrast, no known meristem identity or flowering control gene had, until now, been demonstrated to have a role in ovule development.

A gene termed SHORT INTEGUMENTS1 (*SIN1*), genetically detected in the model plant *Arabidopsis thaliana* by mutational studies, has been determined to be an important regulatory gene for plant reproductive development. The *SIN1* gene is required for normal ovule development. The original isolate of the *sin1* mutation (*sin1-1* allele) was identified as one causing a female sterile phenotype. Ovules of the original isolate have short integuments and a defective megagametophyte. It has been shown that the originally described $Sin1^-$ mutant phenotype is a result of an interaction between *sin1*, and *mod1*, its recessive *mod*ifier, and that *mod1* is *erecta*, a mutation in a putative serine-threonine receptor protein kinase gene. The *sin1-1* or *sin1-2* mutation acting alone causes a defect in the coordination of growth of the two sheets of cells of the inner and outer integuments. All other originally described effects on the ovule, such as the lack of outer integument cell expansion and arrest of the megagametophyte, are due to secondary genetic interactions with *erecta*. There are several prospective protein phosphorylation sites within the SIN1 protein, and these might be substrates of protein kinases, such as the ERECTA product.

In plants homozygous for the weaker *sin1-2* mutant allele, approximately 40% of all ovules in any flower mature into seeds. But these seeds frequently contain embryos arrested at different stages of development, some of which germinate to produce abnormal seedlings. Genetic analysis shows that the maternal expression of the *SIN1* gene is necessary for embryo development.

Not only does this gene function in the formation of seeds, *SIN1* is the only identified plant gene whose maternal expression is important for pattern formation in the zygotic embryo. Both *sin1-1* and *sin1-2* alleles have the maternal-effect embryonic lethality phenotype. The wild type *SIN1* allele when transmitted through the pollen is unable to rescue the deleterious effects on embryogenesis of a homozygous maternal *sin1-2* mutation. Ray et al., "Maternal Effects of the *Short Integument* Mutation on Embryo Development in *Arabidopsis*," Dev. Biol. 180:365-369 (1996) shows that a wild type allele of *SIN1* in the endosperm cannot rescue the maternal-effect of *sin1-2*. This is the first demonstration of a maternal effect embryonic pattern formation gene in a plant.

In *Arabidopsis thaliana,* meristem development progresses through at least three distinct phases: from vegetative (V) through inflorescence (I) to the floral (F) mode, a process known as the "V $\rightarrow$ I $\rightarrow$ F switch." It has been shown that the *sin1* mutation causes a

defect in the V → I → F switch. *SIN1* is needed for the expression of the early flowering phenotype imparted by a TERMINAL FLOWER1 (*tfl1)* mutation, and *tfl1 sin1* double mutants do not produce pollen. Furthermore, the *sinl-1* allele enhances the effect of an *APETALA1 (ap1)* mutation. Thus, *SIN1* represents a genetic connection between ovule development and control of flowering.

In addition, the function of *SIN1* gene is important for controlling the time to flower, another important agronomic factor because the timing of seed production depends on the flowering time. It has been shown by genetic analysis that the *SIN1* gene regulates the activity of a master switch gene, LEAFY (*LFY*) that controls flowering time in *Arabidopsis thaliana*. The LEAFY gene from *Arabidopsis thaliana* was shown to accelerate the flowering time of aspen (an economically important timber plant) from many years to a few months. Additionally, *sin1* mutants are late flowering due to the production of an excess of vegetative leaves and lateral inflorescence axes before producing the floral primordia, which suggests a role of *SIN1* in meristem fate determination. The ability to improve crop plant production through genetic engineering requires the identification and manipulation of previously unidentified genes that control developmentally important plant processes, including ovule development and flowering in plants.

The present invention is directed to overcoming the deficiencies in the prior art.

The rejection of claims 4, 7, 8, and 18-25 under 35 U.S.C. § 112 (2nd para.) for indefiniteness is respectfully traversed in view of the above amendments.

The rejection of claims 1, 4-15, and 18-25 under 35 U.S.C. § 112 (1st para.) for failure to satisfy the written description requirement is respectfully traversed.

The instant application teaches that the nucleic acid molecule of the present invention encodes a multi-domain protein which includes a bipartite N-terminal nuclear localization signal (NLS), an RNA helicase C motif, two RNase III catalytic domains, a PIMS (for PIWI Middle domain-SHORT INTEGUMENTS1, PIWI being a family of important plant developmental proteins) motif, and two C-terminal repeats of a dsRNA binding domain (page 20, line 16 to line 21). The importance of the multi-domain structure to the function of the nucleic acid molecule of the present invention and its predicted protein, in particular with regard to the RNA helicase domain of the SIN1 protein, are discussed in the instant application at page 32, lines 1-15.

To demonstrate that they were in possession of the claimed invention, applicants submit the accompanying Declaration of Animesh Ray under 37 CFR § 1.132 ("Ray Declaration").

In *Arabidopsis thaliana,* only two genes have been identified whose activities are required in the maternal sporophyte (or female somatic cells) for normal pattern formation during embryo development (Ray Declaration ¶ 6). One gene identified in this class is the wild-type allele of *SHORT INTEGUMENTS1 ("SIN1")* (Id.). It has been demonstrated that maternal activity of the *SIN1* gene of *Arabidopsis thaliana* is essential for embryo pattern formation and viability, and that its post-embryonic activity is required for several processes in reproductive development, including flowering time control and ovule morphogenesis (Id.). Furthermore, applicant Ray has shown that homozygous *sin1* mutants exhibit defects in flowering time, delayed transition between developmental states, and failure to coordinate integument formation in ovules (Id.). Most mutant ovules show uncoordinated growth of both the inner and outer integuments and the nucellus, resulting in the extrusion of the embryo sac, with the egg (Id.). These pleiotropic effects suggest that *SIN1* plays a key role in several developmental processes (Id.).

A 6.2 kilobase pair (kb) cDNA (accession number AF292940) corresponding to the *SIN1* mRNA was isolated from a flower- and seed-enriched cDNA library (Ray Declaration ¶ 7). The cDNA was identified as having a 5,727 bp open reading frame (ORF), a 378 bp 5' untranslated region (UTR), a 74 bp 3' UTR and nine adenines at the 3' end, likely to be from the poly-A tail (Id.). The cDNA sequence confirmed the presence of 19 introns and 20 exons (Id.). Shown in the lower portion of Figure 1 of the present invention (and Figure 1B of Exhibit 3) is the arrangement of functional motifs of the predicted SIN1 protein: a bipartite N-terminal nuclear localization signal (NLS), a DexH box RNA helicase C motif, two RNase III catalytic domains, a PIMS (for PIWI Middle domain-SHORT INTEGUMENTS1, PIWI being a family of important plant developmental proteins) domain (identical to the PAZ domain, so called for its occurrence in PIWI, ARGONAUTE, and ZWILLE , all three being developmentally important proteins), and two C-terminal repeats of a dsRNA binding domain (Id.). A BLAST search yielded numerous high homology strikes of the above-identified domains of SIN1, shown in Figure 2 of the present application (Id.). Each of the three functional domains is strongly conserved within its own family (Id.). Structural modeling of the wild-type SIN1 and *sin1* mutant proteins indicates that the RNA helicase domain of SIN1 is particularly important for the protein's function (Id.). For

example, applicant Ray mapped both the *sin1-1* and *sin1-2* alleles to the C-terminal region of the helicase domain, having P415S and I431K substitutions, respectively (Id.). Neither amino acid residue had previously been described as crucial for helicase function (Id.). Amino acid sequence alignment and homology modeling, using the yeast translation initiation factor 4A (yIF4A) helicase as the template, suggests a similar structural basis for the effects of both point mutations in *sin1:* both amino acid substitutions map on the same face of the predicted helicase domain (Id.). The biological function of SIN1 protein, as deduced by the mapping of known mutations (with genetic consequences) into the sequenced and cloned *SIN1* gene, was determined to be essential for plant reproduction and survival, and this demonstration represents the first demonstration of biological function for any member of this class of proteins containing a combination of DexH box helicase domain, PAZ domain, RNase III domain, and dsRNA binding domain (Id.).

The importance of the RNA helicase domain in SIN1-like development-related plant genes is further supported by the work of Jacobsen et al., "Disruption of an RNA Helicase/RNA III Gene in *Arabidopsis* Causes Unregulated Cell Division in Floral Meristems," Development 126:5231-5243 (1999) (Ray Declaration ¶ 8). Jacobsen discloses a recessive mutant, *carpel factory ("caf"),* which converts the floral meristems of *Arabidopsis thaliana* to an indeterminate state (Id.). *Caf* mutants produce extra whorls of stamens, and an indefinite number of carpels (Id.). Thus, *CAF* appears to suppress cell division in floral meristems (Id.). Furthermore, the *CAF* gene cloned by Jacobsen encodes a protein with similarities to both DexH/DEAD-box type RNA helicases and RNase III proteins, suggesting a mechanism for control of floral meristem proliferation (Id.). The isolated gene of the present invention is identical to the CAF gene (gi6102609) except for two single base sequencing differences leading to an erroneous designation of one amino acid residue reported in the CAF protein sequence (Id.). The correctness of the SIN1 sequence, and its identity to the CAF gene, were confirmed in applicant Ray's laboratory by re-sequencing the *caf* mutant allele of Jacobsen (Id.). The identity of *SIN1* to the *SUSPENSOR1 (SUS1)* gene, which is essential for embryogenesis was also shown by PCR analysis and mapping of *sus1* mutations (Id.). The presence of the RNA helicase and RNAse III domains suggest that SIN1/SUS1/CAF functions in *Arabidopsis* development through post-transcriptional regulation of specific mRNA molecules (Id.). Extrapolating from the known functions of RNA helicase proteins and RNAse III proteins, SIN1/SUS1/CAF1 may function in plant development in mRNA processing (Id.). Plants may use small dsRNA hairpins (or

their cleaved products) as developmental regulators over long distances in much the same way as small dsRNA fragments of RNA viral genomes induce systemic signaling for defense against viral pathogens (Id.). If RNA is the substrate for the SIN1-like proteins in plants, then a role in binding, cleavage, and subsequent unwinding of double-stranded RNA is implicated, and the RNase III and RNA helicase domains are clearly important in the functioning of the gene (Id.). Movement of a target RNA from the maternal sporophyte into the developing embryo in a *SIN1/SUS1/CAF* dependent manner could also explain the role of the sporophyte in embryogenesis (Id.). The function of SIN1 in transcriptional regulation is further supported by recent studies that implicate 21-25 nt dsRNA molecules in transcriptional repression of transgenic promoter sequences in plants (Id.).

As noted above, recent studies have implicated dsRNA in transcriptional control, which may involve gene silencing, in early developmental stages in plants (Ray Declaration ¶ 9). Given the molecular nature of the predicted protein product of SIN1 nucleic acid molecule, it is likely that the role of *SIN1* in early embryogenesis is to down regulate the activity of RNA targets required for early embryogenesis (Id.). Effects of the mutant alleles of *SIN1* are entirely consistent with such a function, and *sin1* point mutations, which applicant Ray has mapped to the helicase C domain of the protein, establish the critical requirement of a functional RNA helicase domain for the SIN1 protein (Id.). It is highly likely that the conserved multi-domain structure of SIN1, taught in the present application, is found in early stage development-related genes throughout the plant world (Id.). Armed with the nucleotide sequence and the structural/functional description of SIN1 as taught in the present application, it would be possible for a researcher to obtain a *SIN1*-like gene from any other plant (Id.).

Given all the foregoing, applicants submit that a skilled artisan, having read the instant application, would recognize that the applicants were in possession of the claimed invention. Accordingly, the rejection of claims 1, 4-15, and 18-25 under 35 U.S.C. § 112 (1st para.) for failure to satisfy the written description requirement should be withdrawn.

The rejection of claims 1-15, and 18-25 under 35 U.S.C. § 112 (1st para.) for lack of enablement is respectfully traversed.

It is the position of the U.S. Patent and Trademark Office ("PTO") that the present application is not enabling for any nucleic acid molecule other than that having SEQ ID NO: 1. Applicants respectfully disagree. Applicants submit that one of ordinary skill in the art, using the disclosure of the present application in combination with information well

known to those in the art at the time of filing the instant application, would have been fully able to make and use the claimed invention.

The present invention teaches in detail the cloning, sequencing, and characterization of a *SIN1* gene and its encoded protein product from *Arabidopsis thaliana* (page 6, line 24 to page 21, line 19). The present invention also teaches making a nucleic acid construct having an exemplary SIN1 nucleic acid molecule of the present invention in the sense or the antisense orientation, operably linked to suitable 5' promoter and 3' terminator regions for proper transcription and translation; cloning the nucleic acid construct into an expression vector; introducing recombinant molecules into a host cells to generate transgenic cells; selecting transformants harboring the transgene; re-generating and cultivating transgenic plants; and harvesting transgenic seeds (page 21, line 20 to page 29, line 8). Furthermore, applicants submit that those skilled in the art are familiar with a multitude of protocols for preparing nucleic acid constructs, producing host cells transformed with the nucleic acid construct, and producing transgenic plants and seeds. Several well-known manuals describe molecular cloning techniques, including Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Press, NY (1989), and Ausubel, F. M. et al. (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y.

This is further demonstrated by the accompanying Ray Declaration. Briefly, the Ray Declaration, as well as the exhibits referenced therein, confirm that nucleic acid constructs, expression vectors, host cells, and transgenic plants having a nucleic acid molecule encoding a short integuments1 protein can be produced in accordance with the present application. As described above, one of ordinary skill in the art would have been fully able to obtain other *SIN1*-like gene from the disclosure of the present application. Based on work with transgenic plants according to the present application, the Ray Declaration shows the present application enables one of ordinary skill in the art to carry out the methods of modulating time of flowering, fertility, and fecundity in plants with a SIN1 nucleic acid molecule, in accordance with the present application.

The 6.2-kb full-length *SIN1* cDNA was placed under the constitutive Cauliflower Mosaic Virus 35S promoter (35S::*SIN1*) and the resulting construct (with kanamycin resistance marker for selection) was introduced into otherwise wild type *Arabidopsis thaliana* (Columbia ecotype) plants through *Agrobacterium*-mediated transformation using infiltration methods (Ray Declaration ¶ 10). The resulting

transformants were selected on plates containing kanamycin (Ray Declaration ¶ 11). The presence of the *SIN1* transgene was confirmed by PCR analysis, and PCR positive plants were self-crossed to make lines homozygous for the 35S::SIN1 transgene (Id.). Putative homozygous lines were subsequently confirmed by segregation analysis (Id.). Two of these homozygous transgenic lines clearly expressed the SIN1 protein, as determined by immunocytochemical staining of ovule and meristem sections (see lines 9 and 12 of Figure 3, showing transcript localization via *in situ* hybridization, in Exhibit 11; and Figure 4, showing protein localization via α-SIN1 antibody, in Exhibit 12) (Id.).

As seen in Table 1 (Exhibit 13), the *SIN1* transgene accelerated the time of change to the reproductive phase in two homozygous plant lines (Ray Declaration ¶ 12). Expression of *SIN1* transgene, therefore, promoted early flowering, as assayed by the number of days it took the plants to bolt, as well as decreased the number of leaves produced per plant (Id.). All the transgenic plant lines produced significantly fewer leaves than non-transformed controls, indicating possible subtle effects on flowering time not measurable by applicants' techniques with this model plant (Id.). Unlike 35S::*LFY*, the lateral branches do not terminate into flowers, and single flowers do not develop in the axils of the rosette leaves, consistent with the fact that *SIN1*, unlike *LFY*, is not a floral meristem identity gene (Id.). Thus, *SIN1* is necessary for the vegetative to flowering transition, and expression from a transgene can independently promote flowering in *Arabidopsis* (Id.). This early flowering effect of SIN1 without additional effects on branching pattern is likely to be useful in crop engineering, because other early flowering genes, including *LFY* and *APETALA1*, that are in current use for developing early flowering crop engineering alter branching patterns and reduce the number of flower-bearing branches (Id.).

With regard to the claimed methods of modulating fertility and fecundity with the nucleic acid molecule of the present invention, applicants submit that both methods are enabled by the disclosure of the present invention, as demonstrated by the Ray Declaration. Fertility can be functionally defined as onset of reproductive maturity (Ray Declaration ¶ 13.) Flowering of plants is a measure of the onset of maturity, therefore, plants that flower sooner reach maturity sooner, which results in increased fertility (Id.). When plants were transformed with the 35S::*SIN1* expression construct (6.2 kb cDNA under the control of a 35S promoter), SIN1 transgenic plants were shown to have a shorter time to floral production than control plants (Id.). This demonstrates that the SIN1 nucleic acid molecule of the present application can be used to modulate fertility in plants (Id.).

Fecundity relates to reproductive maturity in combination with the total number of seeds a mature plant can produce (Ray Declaration ¶ 14). Thus, decreasing the time to flowering with expression of the protein of the present invention is one factor of increased fecundity, as it increases time spent in the adult phase (Id.). The other factor, seed development, is also related to expression of the protein of the present invention, as this protein, when maternally expressed, appears to coordinate the expression of zygotic pattern formation in the embryo (Id.). *SIN1* transgenic plants that over-express SIN1 exhibit early flowering and decreased leave production (Id.). This result demonstrates that fecundity can be regulated by use of the *SIN1* transgene in plants as taught in the present invention (Id.).

Accordingly, the rejection of claims 1-15 and 18-25 under 35 U.S.C. § 112 (1st para.) for lack of enablement should be withdrawn.

The rejection of claims 1-3 and 5-6 under 35 U.S.C. § 102 (a) as anticipated by the sequences deposited as Accession number AC007323, submitted April 17, 1999, by J.R. Ecker to the *Arabidopsis thaliana* Genome Center, Department of Biology, University of Pennsylvania ("Ecker") is respectfully traversed.

As evidence that this rejection is improper, applicants submit the Declaration of Teresa A. Golden and Animesh Ray under 37 C.F.R. § 1.131 ("Golden-Ray Declaration"). In particular, NCBI, the publisher of GenBank accession submissions, advised applicants that accession number AC007323 was released to the public on April 19, 1999 (Golden-Ray Declaration ¶ 3).

Furthermore, prior to April 19, 1999, applicants conceived and reduced to practice in the United States the full-length cDNA sequence (6184 b.p.) of the Short Integuments1 ("*SIN1*") gene and the corresponding amino acid sequence of the SIN1 protein encoded by that gene, which applicants cloned from *Arabidopsis thaliana* (Golden-Ray Declaration ¶ 4). Evidence of this reduction to practice is found in attached Exhibit 1, which is a true copy of applicants' laboratory records, showing the DNA Strider™ sequence record of the *SIN1* gene, and the predicted SIN1 amino acid sequence (Id.).

In view of the above, applicants submit that Ecker does not anticipate the present invention. Therefore, the rejection of claims 1-3 and 5-6 under 35 U.S.C. § 102 (a) is improper and should be withdrawn.

In view of all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

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9/30/02	Jo Ann Whalen
Date	Jo Ann Whalen

Appendix A

Version With Markings to Show Changes Made

In reference to the amendments made herein to claims 4, 7, 8, 9, 18, 20, 22, 24, and 25, additions appear as underlined text, while deletions appear as bracketed text, as indicated below:

In The Claims:

4. (Amended) An antisense nucleic acid molecule encoding a nucleic acid sequence which is complementary to the nucleic acid molecule [DNA] according to claim 1.

7. (Amended) An expression vector comprising a transcriptional and translational regulatory DNA operably linked to the nucleic acid [a DNA] molecule according to claim 1.

8. (Amended) An expression vector according to claim 7, wherein the nucleic acid [DNA] molecule is in proper sense orientation and correct reading frame.

9. (Amended) A host cell transduced with the nucleic acid molecule according to claim 1.

18. (Amended) A method of regulating flowering in plants comprising: transducing a plant with the nucleic acid [a DNA] molecule according to claim 1 under conditions effective to regulate flowering in the plant.

20. (Amended) A method of increasing fertility in plants comprising: transducing a plant with the nucleic acid [a DNA] molecule according to claim 1 under conditions effective to increase fertility in the plant.

22. (Amended) A method of increasing fecundity of plants comprising: transducing a plant with the nucleic acid [a DNA] molecule according to claim 1 under conditions effective to increase fecundity of the plant.

24. (Amended) A method of decreasing fertility in plants comprising:

Appendix A

Version With Markings to Show Changes Made

transducing a plant with the nucleic acid [a DNA] molecule according to claim 1 mutated to cause disruption of the nucleic acid [DNA] molecule under conditions effective to decrease fertility.

25. (Amended) A method according to claim 24, wherein the [a] plant is transduced with a nucleic acid [DNA] molecule which encodes either 1) an antisense nucleic acid complementary to the nucleic acid molecule that encodes an amino acid having SEQ. ID. No. 2, 2) an antisense nucleic acid complementary to the nucleotide sequence of SEQ. ID. No. 1, 3) an antisense nucleic acid complementary to a nucleic acid molecule that is at least 55% similar to the nucleotide sequence of SEQ. ID. No. 1 by basic BLAST using default parameters analysis, or 4) hybridizes to the nucleotide sequence of SEQ. ID. No. 1 under stringent conditions characterized by a hybridization buffer comprising 0.9M sodium citrate buffer at a temperature of 45°C.